REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. EXAMINER INTERVIEW, CLAIM STATUS & AMENDMENTS

Applicants thank Examiner Tungaturthi and his supervisor for the telephone interview on November 5, 2007. The claims have been amended along the lines discussed in the interview.

Claims 1, 2, 4-13 and 16-27 were pending in this application when last examined.

Claims 1, 2, 4-13, 18, 19 and 27 were examined on the merits and stand rejected.

Claims 16, 17 and 20-26 were withdrawn as non-elected subject matter

Claim 1 is amended to specify that immunization occurs with a plurality of purified candidate antigens. Claims 1 and 27 are also amended to clarify that the protein chips display purified candidate antigens. Support can be found in the disclosure, for example, at page 1, lines 1-3, page 2, lines 20-27, page 3, lines 20-29, page 4, lines 20-27, page 13, line 27 to page 14, line 4, and original claims 1, 3 and 14.

Claim 6 is amended to include a comma before "wherein" to better conform with US practice and English form.

No new matter has been added.

Claims 18 and 19 have been canceled without prejudice or disclaimer thereto. Applicants reserve the right to file a divisional or continuation application on any canceled subject matter.

Claims 1, 2, 4-13, 16, 17 and 20-27 are pending upon entry of this amendment.

II. OBVIOUSNESS REJECTION

In item 17 on pages 4-20 of the Office Action, claims 1, 2, 4-13, 18, 19 and 27 were newly rejected under 35 U.S.C. § 103(a) as obvious over Mather et al. (WO 2000/037503) in view of Kucherlapati et al. (US 6,150,584), van de Winkel et al. (US2003/0138421, published July 24, 2003), Rava et al. (US 6,720,149), and Kessler et al. (US 2003/0044849). The van de Winkel et al. reference is newly cited.

In the interview, Applicants discussed amending the claims to require immunization with a plurality of purified candidate antigens to overcome the obviousness rejection. In reply, the Examiners questioned whether such amended claims would still be obvious, on the basis that: (1) it appeared that all the elements were disclosed in the art and/or there was motivation to combine/modify the reference teachings to arrive at the claimed invention, and (2) the Examiners questioned whether the isolated whole cell in Mather is equivalent (i.e., would "read on") the "purified candidate antigen" of the amended claimed invention.

In reply to the Office's concerns raised in the interview, Applicants again respectfully submit that the above-discussed amendments overcome the rejection for the following reasons.

The present invention consists in producing multiple monoclonal antibodies against multiple known purified antigens simultaneously by immunizing with **multiple purified** antigens, generating hybridomas and screening the supernatants of the hybridomas on protein chips displaying the antigens.

Amended claim 1 requires immunization of animals with a **plurality** of **purified** antigens, generating immortalized cell lines, screening the supernatant of the cell lines against protein chips on which the **purified** candidate antigens are displayed; and selecting antibodies that bind to the antigens. Kindly note that amended claim 1 corresponds to allowed claim 1 in the equivalent European patent (EP 1 506 235 B1). A copy of the granted claims in the equivalent European patent are attached for the Office's convenience.

Amended claim 27 requires screening the supernatant of immortalized cells lines against one or more protein chips on which **purified** candidate antigens are displayed and selecting monoclonal antibodies that bind the candidate antigens, the method being characterised in that the immortalised cell lines are generated from a single suspension of antibody-producing cells that produce antibodies against a plurality of antigens. This amended claim corresponds to allowed claim 13 of the corresponding European patent.

In the Office Action, Mather was used as the primary reference in the obviousness rejection and it was combined with Kucherlapati, van de Winkel, Rava and Kessler.

Mather relates to a method of generating a group of monoclonal antibodies that are representative of a specific cell type and that will not cross-react with other cells (see abstract). Mather discloses immunizing an animal with **intact** cells and producing hybridomas from the animal (see p17, lines 27-34). See also the paragraph 2 of the Summary of the Invention of Mather. The supernatant of the hybridomas is screened by ELISA against the **intact** cells with which the animal has been immunized to identify a population of monoclonal antibodies characteristic of the cells that have been used to immunize the animal (p18, lines 12-20). The specific target antigens on the cell to which the monoclonal antibodies in this population bind may then be identified, if desired, by immobilizing the antibodies on a column, adding purified antigens and eluting antigens that are bound to the antibodies (p21, l6-10).

Accordingly, Mather does not describe immunization of mammals with purified antigens. Indeed, it is clear from paragraph 3 of the Background of the Invention of the reference, that Mather considers extraction of purified antigens for use as immunogens to be **disadvantageous**. In this sense, Mather **teaches away** from the present invention and there would be no motivation to modify the teachings of the combined cited references to arrive at the claimed invention.

Again, Mather differs from the claimed invention in both the immunization method (i.e., **whole cell vs purified candidate antigens**) and in the screening method to isolate monoclonal antibodies (i.e., **ELISA using whole cells vs protein chips displaying purified antigens**).

Further, in Mather, the identity of the antigens on the **whole cells** against which the monoclonal antibodies were being generated was **not** known. The point of Mather is to generate a population of monoclonal antibodies against **unknown** antigens on the **whole cells** with which the animal is being injected. The identity of the antigens on the cell surface to which the population of monoclonal antibodies that are generated by immunization bind is optionally established at a later stage by screening immobilized antibodies against candidate antigens.

Accordingly, it is clear that Mather fails to disclose or suggest immunization of animals with a plurality of **purified** antigens, generating immortalized cell lines, and then screening the supernatant of the cell lines against protein chips on which the **purified** candidate antigens are displayed.

Moreover, there would be no motivation for the skilled person to replace immunization with **whole cells** in Mather with immunization with **purified** candidate antigens, because the skilled person in Mather did not know the identity of the antigens to which monoclonal antibodies were being generated, and would not therefore know what purified antigens to use for immunization in place of the whole cells. The skilled person would not simply select random purified antigens that he thought might be expressed on the surface of the whole cells for immunization since this would not achieve the primary aim in Mather of generating a population of monoclonal antibodies representative of a specific whole cell which does not cross-react with other whole cells. For this same reason, one of ordinary skill in the art would **not** have replaced screening monoclonal antibodies against **whole** cells with screening against **purified** antigens on protein chips.

The remaining cited references fail to make up for the deficiencies in Mather for the following reasons.

Kucherlapati fails to disclose or suggest immunization of animals with a plurality of purified antigens to simultaneously produce monoclonal antibodies against a plurality of purified

antigens. Further, Kucherlapati does not disclose or suggest the identification of monoclonal antibodies against multiple antigens using a protein chip displaying the purified antigens.

Instead, Kucherlapati discloses immunization of a transgenic mouse having a human immune system (Xenomouse) with a **single** purified antigen, not a **plurality** of **purified** candidate antigens. A **single** antigen is used to immunize the transgenic mouse (column 4, lines 41-50), and B cells from the immunized mouse are used to generate hybridomas producing monoclonal antibodies against this single antigen using Kohler & Milstein's standard method (column 7). Monoclonal antibodies binding to the antigen are identified by screening using a sandwich ELISA (column 7).

All of the examples in Kucherlapati describe immunization of the animal with a **single** antigen followed by generation of hybridomas and identification of monoclonal antibodies binding to the antigen using standard ELISAs. In particular, Example 9 (referred to by the Examiner) discloses immunization of the mouse with recombinant IL-8 (column 18, lines 15-20), isolation of spleen cells and generation of hybridomas (column 18, lines 35-50) and identification by ELISA of hybridomas producing monoclonal antibodies that bound IL-8 (column 18, lines 55-62).

Example 9 referred to by the Examiner discloses immunization of the Xenomouse with IL-8 (i.e., a single antigen) and generation of hybridomas from spleen cells. The supernatant of these hybridomas is screened against IL-8 by ELISA (not protein chips) to identify monoclonal antibodies binding IL-8 (col 18, 155-60). This is the step of identifying the monoclonal antibody. Only once the monoclonal antibody binding to IL-8 has been identified by ELISA are further experiments conducted to ascertain the properties of the monoclonal antibody that has been isolated. In one of these experiments, recombinant IL-8 is bound to a gold chip and used to investigate the kinetics of the monoclonal antibody using a BIAcore instrument (col 19, 121-27).

Applicants respectfully submit that the Office is incorrect to suggest that the experiment conducted with the BIAcore instrument corresponds to the claimed step of screening the

supernatant of hybridomas against protein chips displaying purified antigens used for immunization in order to identify antibodies that bind to the protein chip. Instead, in Kucherlapati, the step of screening the supernatant to identify the antibodies that bind antigen was carried out by ELISA.

Therefore, Kucherlapati differs from the claimed methods in two ways: i) it immunizes with a **single** purified antigen and not with multiple purified antigens; and ii) it discloses identifying monoclonal antibodies that bind to the antigen by ELISA with the antigen and **not by screening using a protein chip** displaying the purified antigen.

Kucherlapati focused on the production of a monoclonal antibody to a **single** antigen. As such, there is no motivation in Kucherlapati to replace immunization with one purified antigen with immunization with multiple purified antigens. Nor is there any suggestion to replace ELISA screening with screening against a protein chip to identify multiple monoclonal antibodies simultaneously.

In fact, the data filed by Applicant show that these changes enable high-throughput production of multiple monoclonal antibodies against multiple antigens simultaneously, a possibility that had not been contemplated previously. The present invention also has the advantage that it is possible to map where on an antigen any antibody binds by screening every supernatant against multiple antigens, something that is totally impracticable with immunization with a single antigen and screening by ELISA. In this regard, the present invention exhibits unexpected results over the cited prior art reference. Again, none of the cited references provide motivation for these changes nor do they recognize the improvements associated these changes. Accordingly, Applicants were first to realize that these changes would remove the bottleneck in monoclonal antibody production and allow fast and efficient production of monoclonal antibodies against multiple different antigens.

Accordingly, it is clear that Kucherlapati does **not** describe immunization of animals with a **plurality** of **purified** antigens in order to produce monoclonal antibodies against a plurality of

purified antigens simultaneously. Also, Kucherlapati does not describe the identification of monoclonal antibodies against multiple antigens using a protein chip displaying the purified antigen.

For these reasons, the invention of the amended claims is novel and non-obvious over the combination of Mather and Kucherlapati.

Van de Winkel fails to remedy the deficiencies of Mather and Kucherlapati.

The disclosure of van de Winkel is similar to Kucherlapati in that it also discloses immunization with a **single** purified antigen (IL-8) to produce monoclonal antibodies that bind to IL-8 and identification of these antibodies by ELISA using IL-8. Accordingly, the comments relating to Kucherlapati are also applicable to van de Winkel.

Rava describes a biological chip plate for conducting multiple biological assays. The plate contains a plurality of wells, each well containing a chip having a molecular probe array. The focus of Rava is on the production of plates containing DNA arrays for use in detecting DNA or RNA molecules. The Applicant does not dispute that protein chips were available before the filing date, nor that these chips could be used to detect a monoclonal antibody to a particular antigen.

However, there is no discussion at all in Rava of the production of monoclonal antibodies. There is also no suggestion in Rava that a plurality of purified antigens could be displayed on chips simultaneously to detect monoclonal antibodies that bind to a plurality of different antigens. Thus, it is clear that Rava fails to remedy the above-noted deficiencies of Mather and Kucherlapati.

Again, prior to the current invention, no one had realized that vast improvements in terms of speed and efficiency in monoclonal antibody production could be achieved by immunizing with **multiple purified** antigens and then screening with protein chips displaying the **purified** antigens. Where it was desired to produce monoclonal antibodies against known antigens, the standard practice was to follow established methods of immunizing with a single antigen and

then screening using an ELISA – this is the situation disclosed in van de Winkel and Kucherlapati.

Therefore, there is no motivation in van de Winkel or Kucherlapati to alter this standard practice and screen supernatant using the protein chips disclosed in Rava, nor to immunize with multiple antigens. Mather does not provide motivation for immunization with multiple antigens, since it relates to immunization with unknown antigens using whole cells, not to the production of monoclonal antibodies against known purified antigens.

Kessler relates to isotyping of monoclonal antibodies. This paper appears to have been cited by the Office as relevant to dependent claims relating to isotyping. As discussed in the last response, Kessler is concerned with methods of producing antibodies by immunization with whole cells or heterogeneous mixtures. There is no teaching or suggestion in Kessler that an animal or animals can be immunized with a plurality of purified antigens to produce monoclonal antibodies against a plurality of purified antigens. Nor is there any suggestion the resulting monoclonal antibodies be screened using protein chips displaying multiple purified antigens.

For these reasons, the cited combination fails to disclose or suggest each and every element of the claimed invention.

The above amendments and arguments were presented in the interview. The Examiners indicated a willingness to seriously consider whether such amendments and arguments overcome the remaining obviousness rejection. The Examiners seemed to agree that such an amendment should overcome the prior art rejection of record, if the isolated whole cell in Mather does not read on the "purified candidate antigen" of the invention (i.e., issue 2 above). This regard, the Examiners questioned whether the term "purified candidate antigen" of the invention could be broadly construed to read on a "whole cell."

In reply, Applicants submit that one of skill in the art, based on art recognized definitions, would clearly understand that a "purified candidate antigen" is **not equivalent** to a whole intact cell. In this regard, kindly note the definition of "purified candidate antigen" at page 3, lines 9+

of the Specification. This definition distinguishes "purified candidate antigen" of the claimed invention from "whole cell" of the cited references.

On page 15 of the Office Action, it was again argued that the arguments in the last response were unpersuasive, because Applicants argued the references individually, rather than focusing on the combination. Applicants again respectfully disagree. It appears that the Office has overlooked the arguments regarding the **teaching away** in Mather from the combination. As discussed above, Mather considers extraction of purified antigens for use as immunogens to be **disadvantageous**. In this sense, Mather **teaches away** from the present invention, which requires purified antigen.

It is well established that there is no motivation to combine/modify references where the cited references teach away from the combination. The prior art must be considered in its entirety and that references cannot be combined where the references teach away from their combination. See M.P.E.P. § 2145 X, D, 2. A reference can be said to teach away when a person of ordinary skill in the art, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path taken by the applicant or if it suggests that the line of development flowing from the reference's disclosure is unlikely to be productive of the result sought by the applicant.

In view of the teaching away in Mather, the cited references cannot be combined/modified to arrive at the claimed invention. Accordingly, there would be no reasonable expectation of success of combining/modifying the cited art teachings to arrive at the use of purified antigens as immunogens as in the present invention, because Mather discloses that it would disadvantageous to do so.

In summary, none of the references cited by the Office suggest immunizing an animal or animals with a plurality of purified candidate antigens in order to produce monoclonal antibodies against all of these antigens. In addition, none of the cited references suggest using protein chips displaying a plurality of purified antigens to screen for monoclonal antibodies that bind to these

antigens. In fact, the presence of both of these steps in a single method results in enormous advantages over conventional methods of producing a single monoclonal antibody by immunizing an animal with a single antigen, producing hybridoma cells using Kohler & Milstein's method and identifying monoclonal antibodies against the antigen using an ELISA.

As evidence of these advantages, Applicants again point to the De Masi et al. paper (published by the inventors) attached to the last response. This paper presents evidence of **unexpected results** for the present invention.

Again, the claimed methods enable the production and screening of monoclonal antibodies against large numbers of purified antigens simultaneously. This advantage alone represents huge progress over methods of generating and screening monoclonal antibodies against a single antigen, as disclosed for example, in Kucherlapati. The present invention also enables huge numbers of hybridoma cell supernatants to be screened compared to prior art methods, making it more efficient at identifying high affinity antibodies. The claimed methods are simple and many times faster than conventional methods of the type disclosed in the cited references. The methods claimed are also more economical due to the fact that they require hardly any antigen for use in the screening procedure.

There is no suggestion in any of the other documents cited by the Examiner that an improved method for producing and screening monoclonal antibodies which enables the simultaneous and efficient production of monoclonal antibodies against a plurality of purified candidate antigens could be obtained by immunizing an animal or animals with more than one purified antigen and by screening the monoclonal antibodies produced using protein chips. It might well have been obvious to the skilled person at the priority date that it was desirable to achieve the advantages provided by the methods of the invention but it would not have been obvious how to achieve them.

It is respectfully submitted that such advantageous and unexpected results are indicative of the nonobviousness of the present invention.

Therefore, the above-noted 103(a) obviousness rejection is untenable and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

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ATTACHMENT

1. Copy of front page and claims of equivalent European patent (EP 1 506 235 B1)

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(54) METHOD FOR PRODUCING MONOCLONAL ANTIBODIES

METHODE ZUR PRODUKTION MONOKLONALER ANTIKÖRPER

PROCEDE DE PRODUCTION D'ANTICORPS MONOCLONAUX

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(56) References cited:
WO-A-01/14425 **WO-A-96/33735**
WO-A-03/019192

- **TEMPLIN M F ET AL: "Protein microarray technology" TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 20, no. 4, 1 April 2002 (2002-04-01), pages 160-166, XP004344214 ISSN: 0167-7799**
- **LUEKING A ET AL: "PROTEIN MICROARRAYS FOR GENE EXPRESSION AND ANTIBODY SCREENING" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 270, no. 1, May 1999 (1999-05), pages 103-111, XP000949937 ISSN: 0003-2697**
- **HAAB B B ET AL: "PROTEIN MICROARRAYS FOR HIGHLY PARALLEL DETECTION AND QUANTITATION OF SPECIFIC PROTENS AND ANTIBODIES IN COMPLEX SOLUTIONS" GENOME BIOLOGY (ONLINE), XX, GB, vol. 2, no. 2, 2001, page COMPLETE XP001147826 ISSN: 1465-6914**
- **ARENKOV P ET AL: "Protein microchips: use for immunoassay and enzymatic reactions" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 278, no. 2, 15 February 2000 (2000-02-15), pages 123-131, XP002234264 ISSN: 0003-2697**
- **EKINS R P: "LIGAND ASSAYS: FROM ELECTROPHORESIS TO MINIATURIZED MICROARRAYS" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 44, no. 9, 1998, pages 2015-2030, XP002939389 ISSN: 0009-9147**

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The file contains technical information submitted after the application was filed and not included in this specification

EP 1 506 235 B1

tified by ELISA suggests that microarray screening is more sensitive than ELISA. Microarray screening further had the significant advantage that it allowed simultaneous determination of the IG or IgM isotype of the monoclonal antibodies identified.

[0108] Figure 3A shows the normalised values of percentage contribution to total intensity for each culture supernatant in an ELISA plate (■) containing positive samples that bind B5 compared to the normalised values of percentage contribution for the same culture supernatants obtained by microarray screening of a B5-coated slide (□). Figure 3B shows the level of background noise in these experiments. It can be seen that positive supernatants showed a greater percentage contribution to total intensity using microarray screening compared to ELISA. As a result, there was a greater difference between background noise and a positive supernatant in microarray screening compared to ELISA, enabling positive supernatants to be identified more easily and more accurately.

[0109] Figure 4A compares the normalised values of percentage contribution to total intensity for each culture supernatant in an ELISA plate (■) containing a single positive sample that binds B5 compared to the normalised values of percentage contribution for the same culture supernatants obtained by microarray screening on a B5-coated slide (□). The level of background noise is shown in Figure 4B and it can be seen that positive sample was more readily detectable above the background noise using microarray screening compared to ELISA.

[0110] Figure 5A compares the normalised values of percentage contribution to total intensity for each culture supernatant in the ELISA plate found to contain positive supernatants that bind KET94/95 (■) compared to the normalised values of percentage contribution for the same culture supernatants obtained by microarray screening on a KET94/95-coated slide (□). The positive supernatants were more readily detectable above the background noise using microarray screening compared to ELISA, as shown in Figure 5B.

[0111] Figures 6A compares the data obtained from an ELISA plate (■) in which there were no positive supernatants to data obtained using microarray screening (□) of the same culture supernatants. As shown in Figure 6B, the readings in both cases were due to background noise.

[0112] These results demonstrate that the method of the invention can be used to simultaneously identify monoclonal antibodies against more than one antigen. The use of microarray screening in the method of the invention is quicker, cheaper and more accurate than the use of conventional antibody screening methods, such as ELISA.

References

[0113]

Kilpatrick *et al* (1997) Hybridoma 16: 381-389

Kohler G, Milstein C (1975) Nature 7: 256:485-7

Lindley et al (2000) J Immunol Methods 234: 123-135

Ziaudin J, Sabatini D (2001) Nature 411: 107-110

Zimmermann U. (1990) J Immunol Methods Nov 6; 134(1):43-50

Claims

1. A high-throughput method of producing a plurality of monoclonal antibodies, each of which binds to a different candidate antigen, said method comprising the steps of:

   a) introducing a plurality of purified candidate antigens into an non-human animal or non-human animals;
   b) recovering antibody-producing cells from said animal or animals and rendering these cells into a single cell suspension;
   c) generating immortalized cell lines from said single cell suspension;
   d) screening the supernatants of said immortalized cell lines against a protein chip on which the candidate antigens are displayed; and
   e) selecting monoclonal antibodies that bind to said candidate antigens.

2. The method of claim 1 wherein said animal is a mouse, a rat, a guinea pig or a rabbit.

3. The method of claim 1 or claims 2 wherein between two and fifty different purified candidate antigens are introduced into the animal or animals.

4. The method of claim 3 wherein between 0.001 and 1000 micrograms of each antigen is introduced into the animal or animals.

5. The method of any one of claims 1 to 4 comprising the additional step of supplying the animal or animals with a booster dose of some or all of the antigens which were introduced into the animal or animals prior to the removal of antibody-producing cells.

6. The method of any one of claims 1 to 5 wherein the antibody-producing cells are B cells, T cell or stem cells.

7. The method of any one of claims 1 to 6 wherein the antibody-producing cells are recovered by removal of spleen tissue, lymph nodes or bone marrow of the animal or animals.

8. The method of any one of claims 1 to 7 wherein the immortalized cell line is a hybridoma cell line produced by somatic fusion of the cells in the single cell

suspension to myeloma cells.

9. The method of any one of claims 1 to 8 wherein said protein chip is a plain-glass slide, a 3D gel pad chip, a microwell chip or a cell chip.

10. The method of any one of claims 1 to 9 wherein the step of detecting the monoclonal antibodies bound to the antigens further comprises isotyping the monoclonal antibodies.

11. The method of claim 10 wherein said step of detecting and isotyping the monoclonal antibodies comprises adding isotype specific anti-immunoglobulin antibodies to said protein chip, wherein each anti-Immunoglobulin antibody having a different isotype specificity has a different label, and detecting the presence of said labels.

12. The method of any one of claims 1 to 11 further comprising assessing the specificity with which each isolated monoclonal antibody binds to an antigen using a protein chip comprising said antigen.

13. A method of identifying a plurality of monoclonal antibodies, each of which binds to a different candidate antigen, said method comprising the steps of:

    a) screening the supernatant of immortalised cell lines against one or more protein chips on which the candidate antigens are displayed; and
    b) selecting as said monoclonal antibodies, antibodies that bind to said candidate antigens,

said method being **characterised in that** said immortalised cell lines are generated from a single suspension of antibody-producing cells that produce antibodies against a plurality of antigens.

14. A method of producing a bank of immortalised cell lines that produce a plurality of monoclonal antibodies of interest, each of which binds to a different candidate antigen, said method comprising the steps of:

    a) introducing a plurality of different antigens into an non-human animal or non-human animals;
    b) recovering antibody-producing cells from said animal or animals and rendering these cells into a single cell suspension;
    c) generating immortalized cell lines from said single cell suspension;
    d) screening the supernatant of said immortalized cell lines against a protein chip on which the candidate antigens are displayed; and
    e) selecting as an immortalised cell lines, that which produce supernatants containing antibodies that binds to said candidate antigens.

Patentansprüche

1. Hochdurchsatzverfahren zur Herstellung einer Vielzahl monoklonaler Antikörper, von denen jeder an einen verschiedenen Antigenkandidaten bindet, wobei das Verfahren die Schritte umfasst:

    a) das Einführen einer Vielzahl gereinigter Antigenkandidaten in ein nicht humanes Tier oder nicht humane Tiere,
    b) das Rückgewinnen Antikörper-produzierender Zellen aus dem Tier oder den Tieren und das Vereinen dieser Zellen in eine Einzelzellsuspension,
    c) das Erzeugen immortalisierter Zelllinien aus der Einzelzellsuspension,
    d) das Screenen des Überstands der immortalisierten Zelllinien gegen einen Protein-Chip, auf welchem die Antigenkandidaten dargestellt sind, und
    e) das Auswählen monoklonaler Antikörper, welche an die Antigenkandidaten binden.

2. Verfahren nach Anspruch 1, wobei das Tier eine Maus, eine Ratte, ein Meerschweinchen oder ein Kaninchen ist.

3. Verfahren nach Anspruch 1 oder Anspruch 2, wobei zwischen zwei und fünfzig verschiedene gereinigte Antigenkandidaten in das Tier oder die Tiere eingeführt werden.

4. Verfahren nach Anspruch 3, wobei zwischen 0,001 und 1000 Mikrogramm jedes Antigens in das Tier oder die Tiere eingeführt werden.

5. Verfahren nach einem der Ansprüche 1 bis 4, umfassend den zusätzlichen Schritt des Versorgens des Tieres oder der Tiere mit einer Boosterdosis von einigen oder allen der Antigene, welche vor dem Entfernen Antigenproduzierender Zellen in das Tier oder die Tiere eingeführt wurden.

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei die Antikörperprodzierenden Zellen B-Zellen, T-Zellen oder Stammzellen sind.

7. Verfahren nach einem der Ansprüche 1 bis 6, wobei die Antikörper-produzierenden Zellen durch das Entfernen von Milzgewebe, Lymphknoten oder Knochenmark des Tieres oder der Tiere rückgewonnen werden.

8. Verfahren nach einem der Ansprüche 1 bis 7, wobei die immortalisierte Zelllinie eine Hybridom-Zelllinie ist, welche durch somatische Verschmelzung der Zellen in der Einzelzellsuspension zu Myelomzellen hergestellt wird.